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We have focused on the ability to predict responsiveness to tubulin targeting chemotherapeutic drugs by understanding the role of p53 repression on the expression of proteins that regulate microtubule dynamics. We previously demonstrated that repression of microtubule associated protein 4 (MAP-4) produced tubulin depolymerization, sensitivity to vinca alkaloids, and resistance to taxanes. We built on this observation to design a phase I clinical trial to determine if this could be accomplished in and confirmed the ability to produce this effect in several human specimens. At the same time we studied the repression of stathmin, a cytosolic phosphoprotein which binds to and stabilizes tubulin heterodimers. Stathmin appears to be more sensitive to repression by p53 than MAP-4 and may lead to additional approaches to treatment of patients with breast cancer during the next grant period.

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Table of Contents

INTRODUCTION

We have focused on the ability to predict responsiveness to tubulin targeting chemotherapeutic drugs by understanding the role of p53 repression on the expression of proteins that regulate microtubule dynamics. We previously demonstrated that repression of microtubule associated protein 4 (MAP-4) produced tubulin depolymerization, sensitivity to vinca alkaloids, and resistance to taxanes. We built on this observation to design a phase I clinical trial to determine if this could be accomplished in patients, In the first nine patients treated with doxorubicin to induce DNA damage, stabilize p53, and repress MAP4, we confirmed the ability to produce this effect in several human specimens. In addition, by escalating the doses of the vinca alkaloid, vinorelbine, we will define a Phase II dose for the sequential combination. At the same time we studied the repression of stathmin, a cytosolic phosphoprotein which binds to and stabilizes tubulin heterodimers. Stathmin appears to be more sensitive to repression by p53 than MAP-4 and may lead to additional approaches to treatment of patients with breast cancer during the next grant period.

BODY:

Task 1. To determine whether loss of p53 function leads to increased expression of proteins that regulate microtubule dynamics in human breast cancer.

Task 2. To determine whether overexpression of proteins that regulate microtubule dynamics is predictive of response to tubulin targeting drugs.

We focused our attention during this grant period on the possibility that MAP-4 could be repressed by p53 induction in human breast cancer cell lines and in patients with breast cancer. We found mixed results in that several cell lines did not repress MAP4 following p53 induction by a variety of agents. We took several approaches to explain the variability between cell lines in their ability to repress MAP-4 after stabilization of wt p53. The first was to investigate the role of bcl-2, an antiapoptotic protein shown by Murphy and colleagues to block gene repression by p53 (Murphy et al., 1996). In these studies we first determined whether bcl-2 was overexpressed in cell lines without MAP-4 repression and found that this was generally the case. We next attempted to block the effect of bcl-2 on MAP-4 repression by down regulation of bcl-2 with retinoic acid (Toma et al., 1997), or destruction with bcl-2 antisense. In both cases we were unable to induce MAP-4 repression despite interfering with the function of bcl-2 (Alli, E. et al, unpublished).

During this grant period we translated our findings with MAP-4 into a phase I clinical trial designed to answer the question "does DNA damage induce wild type p53, repress MAP-4, and sensitize cells to vinorelbine". Using escalating doses of doxorubicin to induce DNA damage, we found that it was feasible to carry out this protocol in breast cancer patients and that in several cases the expected biological response was observed

(Bash et al., unpublished). We are nearing completion of this Phase I trial, and plan to enter phase II during the next year.

During this last grant period we also began to investigate the role of stathmin, a second protein regulated by p53, to determine its role in regulating the sensitivity to tubulin targeting drugs. We turned our attention to stathmin because Murphy et al., had shown that this gene was more sensitive to p53 repression than MAP-4. In addition, since stathmin binds to tubulin heterodimers and inhibits microtubule polymerization, the balance between stathmin and MAP-4 could determine the sensitivity of cells to tubulin targeting drugs. Using a panel of human breast cancer cell lines, we found that induction of wild type p53 decreased the cellular content of stathmin and that this response was more predictable than the repression of MAP-4 (appendix 1). In addition, we found that cells with mutant p53 had increased content of stathmin. To investigate the role of stathmin in more detail, we transfected human breast cancer cell lines with a human stathmin expression construct under the control of a CMV promoter and analyzed both sense and antisense transfectants. These studies demonstrated that cell lines with increased stathmin had decreased microtubule polymerization as measured by immunoflourescent staining of β-tubulin. During the next grant period we will use cell lines stably transfected with stathmin to investigate the effect of alterations in stathmin on the sensitivity to tubulin targeting drugs used in the treatment of breast cancer.

Finally, our laboratory discovered that p53 was involved in the multidrug resistance phenotype through a series of experiments demonstrating that wild type p53 transcriptionally repressed *mrp*1, the multidrug resistance protein gene (Sullivan et al., 2000). This observation will now be extended to our studies of human breast cancer to determine whether or not p53 may have even greater effects on drug sensitivity than we initially hypothesized.

KEY RESEARCH ACCOMPLISHMENTS:

- Characterized the expression of stathmin in a panel of human breast cancer cell lines and MAP-4 in several human specimens.
- Demonstrated the ability to repress stathmin in breast cancer cell lines treated with DNA damaging agents.
- Created stathmin transfectants to investigate the role of this p53-regulated protein in the sensitivity to tubulin targeting drugs used to treat breast cancer.
- Discovered that MRP was regulated by p53.

REPORTABLE OUTCOMES:

Manuscripts

DiPaola RS, Rafi MM, Vyas V, Toppmeyer D, Rubin E, Patel G, Goodin S,Medina M, Medina P, Zamek R, Zhang C, White E, Gupta E and Hait WN: Phase I Clinical and Pharmacologic Study of 13-cis-Retinoic Acid, Interferon Alpha, and Paclitaxel in

Patients with Prostate Cancer and Other Advanced Malignancies J Clin Oncol 17:2213-2218, 1999.

Zhang CC, Yang JM, White E, Murphy M, Levine A and Hait WN: DNA damage increases sensitivity to taxanes through p53-dependent repression of microtubule-associated protein-4. Cancer Res 59:3663-3670, 1999.

Yang JM, Yang GY, Medina DJ, Vassil AD, Liao, J and Hait WN: Treatment of multidrug resistant (MDR1) murine leukemia with P-glycoprotein substrates accelerates the course of the disease. Biochemical & Biophysical Res. Communications 266, 167-173 (1999).

Sullivan GF, Yang JM, Vassil A, Yang J, Bash-Babula J, and Hait WN: Regulation of expression of the multidrug resistance protein MRP1 by p53 in human prostate cancer cells. *J Clin Investigation* 105:1261-1267, May 2000.

Abstracts.

Rafi M, Kotenko S, Pestka S, Toppmeyer D, Hait W and DiPaola RS: Cytotoxicity and bcl-2 modulation by Interferon alpha. Proc Amer Cancer Res 40:3179, 1999.

Zhang C, Bash JE and Hait WN: DNA Damaging Agents Increase wild type p53, Suppress Microtubule Associated Protein 4 (MAP4), Sensitize cells to Vinca Alkaloids and Render cells Resistant to Taxanes. Proc Amer Cancer Res 40:633, 1999.

Sullivan GF, Yang JM and Hait WN: Regulation of the expression of multidrug resistance associated protein by p53 in prostate cancer. Proc Amer Cancer Res 40:4397, 1999.

DiPaola RS, Rafi M, Toppmeyer D, Rubin E, Medina P, Goodin S and Hait WN: bcl-2 Modulation with 13-cis retinoic acid, Alpha Interferon and Paclitaxel in vitro and in Patients with Prostate Cancer and Advanced Malignancy. Proc Amer Soc Clin Onc18:1242, 1999.

Bash-Babula JE, Alli EL, Toppmeyer DL, Hait WN: Effect of Doxorubicin Treatment on p53 and Microtubule-Associated Protein 4 (MAP-4) Expression in Patients with Breast Cancer. Proc Amer Cancer Res 41 2124, 2000.

Development of cell lines,

- Created two stathmin transfectants in BT20 cells (Alli et al., unpublished).

Funding applied for based on this work:
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DOD in prostate cancer (in preparation)

CONCLUSIONS:

In conclusion, we have confirmed the importance of the role of proteins that regulate microtubule dynamics in human breast cancer cell lines and specimens and their regulation by p53. We have capitalized on these observations to develop a new clinical trial for patients with breast cancer. We demonstrated the sensitivity of stathmin to p53 repression and for the first time demonstrated the role of p53 in multidrug resistance.

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Appendix I.

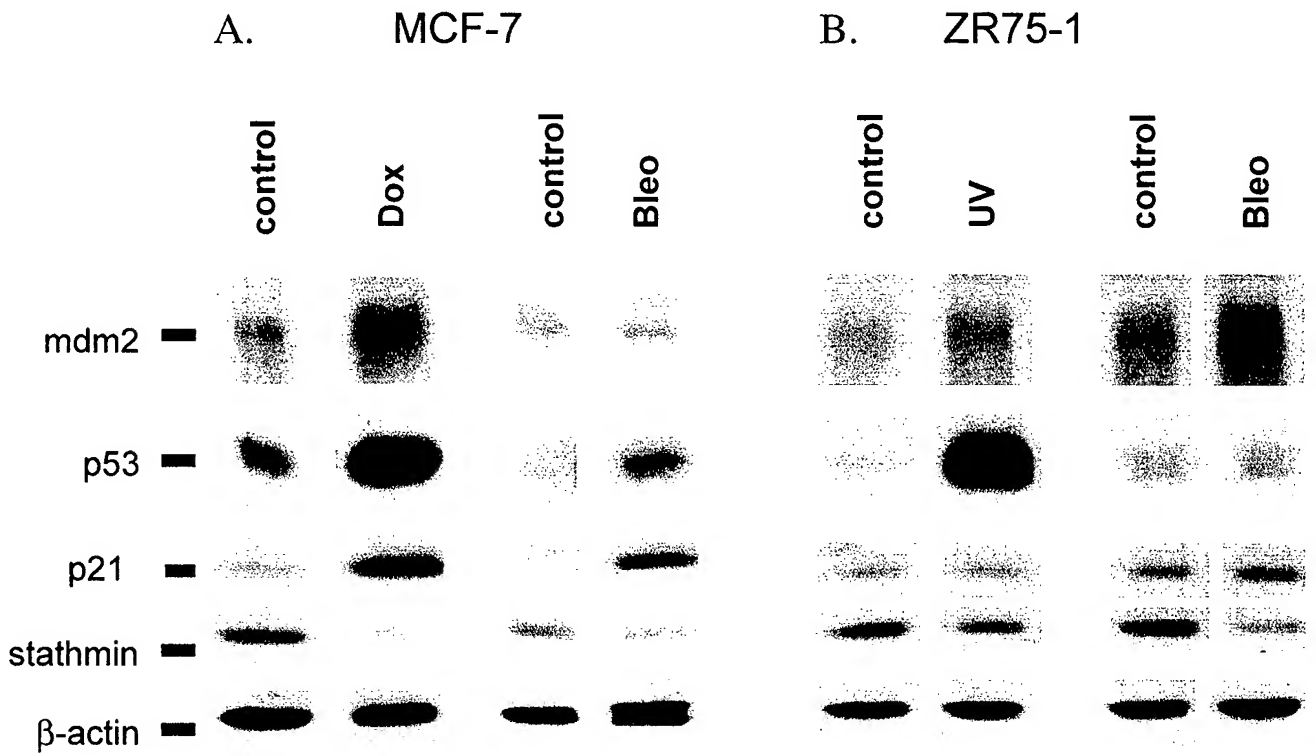

Figure 1. Western blot analysis of p53, mdm2, p21, stathmin and β-actin levels of MCF7 (A) and ZR75-1 (B), two wild-type p53-containing human breast cell lines. MCF-7 cells were left untreated or treated with 1 μM Doxorubicin or 5 μM Bleomycin. ZR75-1 cells were left untreated or treated with 20 seconds of UV-C or 800 nM Bleomycin. 30 μg of protein was loaded to detect p53 and mdm2; 100 μg of protein was loaded to detect p21, stathmin, and β-actin.

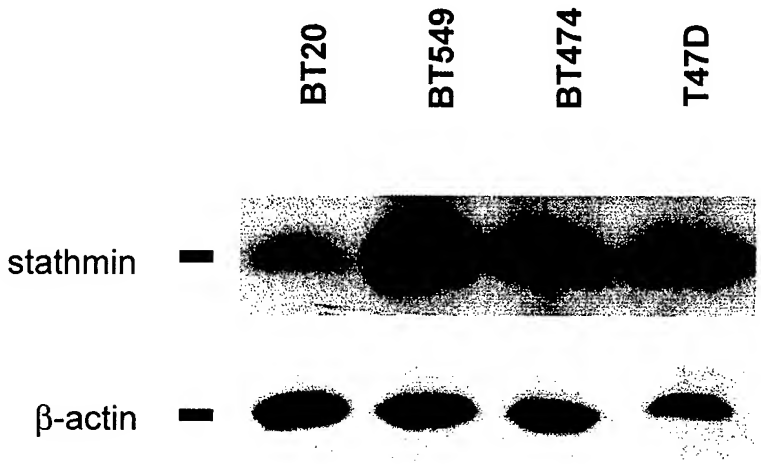

Figure 2. Western blot analysis of stathmin and β-actin levels in mutant p53-containing human breast cell lines. 100 μg of protein was loaded.

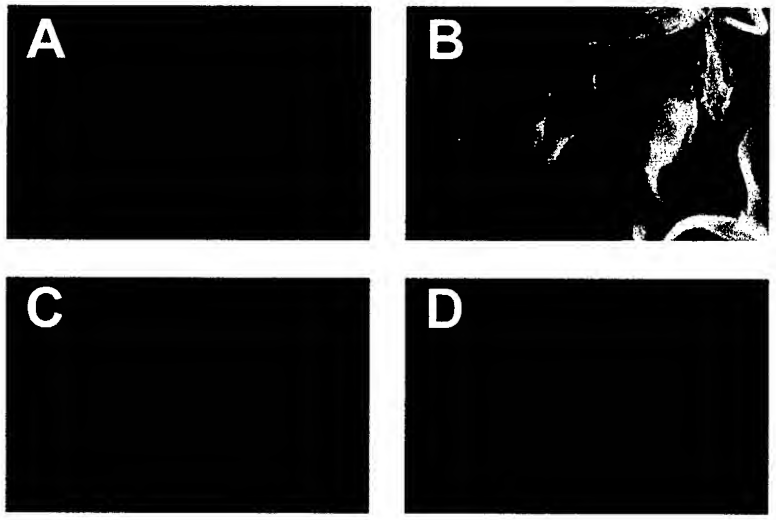

Figure 3. Immunofluorescent staining of stathmin and polymerized microtubules in BT20 (A,B) and BT549 (C,D) cells.
Stathmin, red. Microtubules, green.

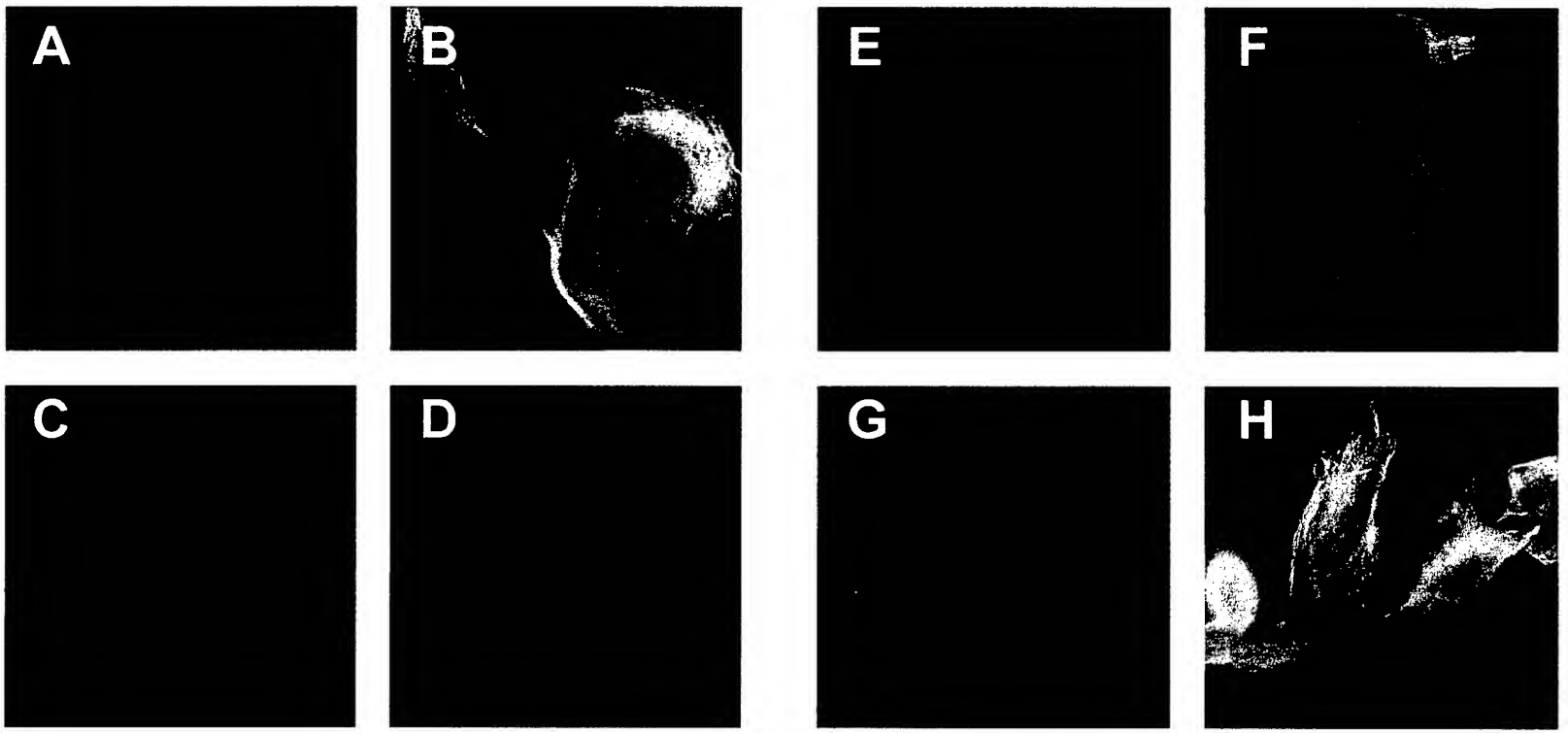

Figure 4. Immunofluorescent staining of stathmin and polymerized microtubules in BT20 vecor alone- transfected cells (A, B), BT20 stathmin transfected cells(C,D), BT549 vector alone- transfected cells (E, F), and BT549 stathmin antisense transfected cells (G, H). Stathmin, red. Microtubules, green.

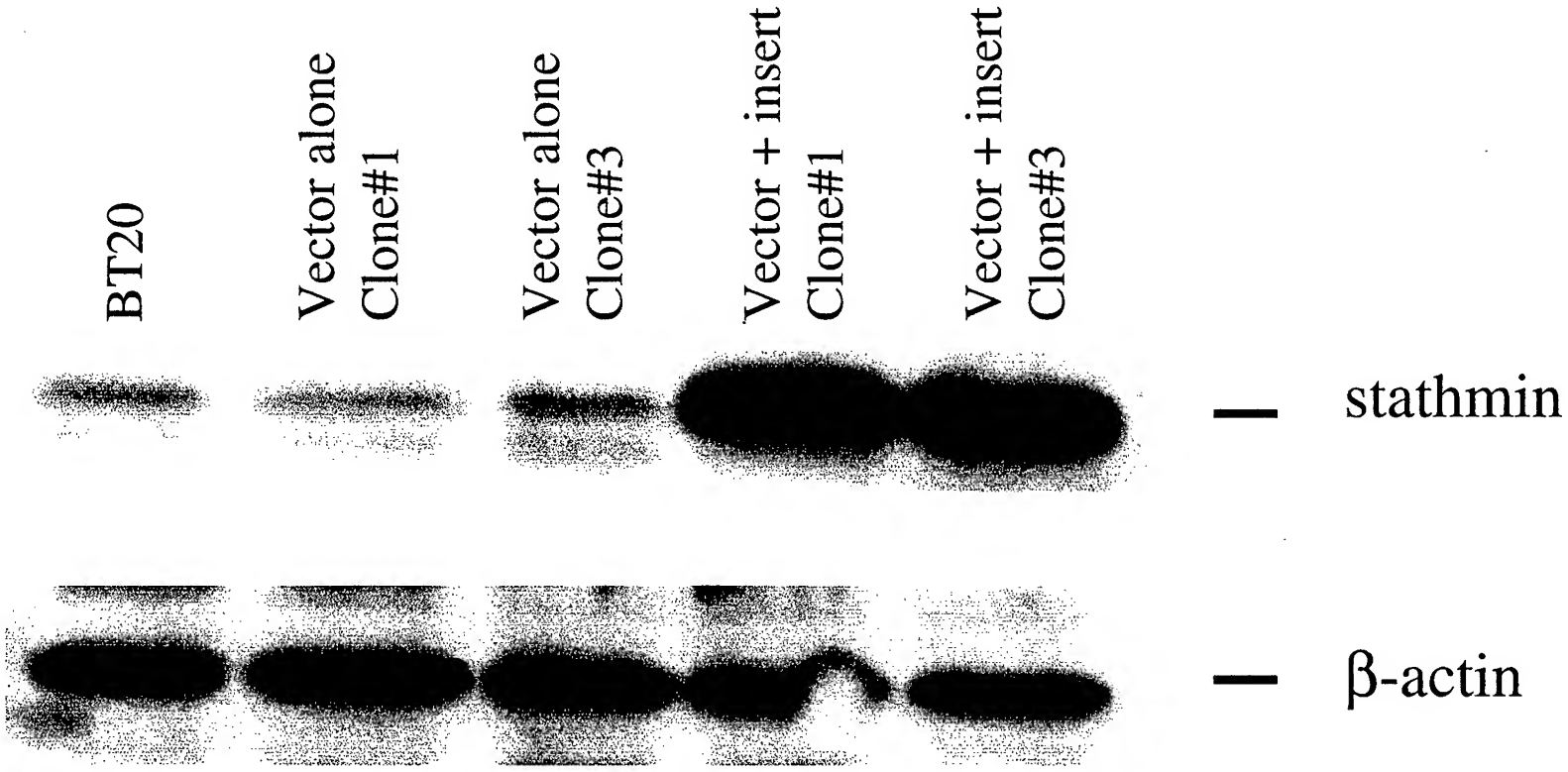

Figure 5. BT20 cells were stably transfected with stathmin. Cells were plated and grown to 90% confluency and transfected with 10 μg of pcDNA3.1 (Invitrogen) containing the full length human stathmin sequence (obtained by Maureen Murphy) or vector alone using Lipofectamine 2000 transfection reagent (Gibco).

Clones were isolated and screened by western blot analysis. 50 μg of protein was loaded.